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Effect of Neurotoxic Pesticides on the Feeding Rate of Marine Mussels (*Mytilus edulis*)†

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Abstract: The effects of selected neurotoxic pesticides on the feeding rate of marine mussels (*Mytilus edulis*) were determined. Two organochlorine pesticides, lindane and endrin, two acetylcholinesterase-inhibiting compounds, dichlorvos and carbaryl and two pyrethroids, flucythrinate and permethrin, were studied. No evidence was found for any specific neurotoxic effect of the organochlorines and pyrethroids on feeding efficiency. In contrast, dichlorvos and carbaryl inhibited the enzyme acetylcholinesterase in mussel gills and were more toxic to feeding efficiency than could be explained by a narcotic mechanism of toxicity alone. Dichlorvos also caused clear behavioural changes in the mussels. The significance of these observations for the application of mussels to impact assessment in the marine environment is discussed.

Key words: mussels, neurotoxic pesticides, feeding rate, QSARs

1 INTRODUCTION

Chemicals which are created or mobilised as a consequence of human activity often become contaminants of estuarine and coastal regions. They may enter these regions *via* direct discharges from ships and adjacent urban, industrial and agricultural activities or be carried over large distances from inland sources through atmospheric or riverine transport. The spatial distribution, severity and biological impact of this contamination has been successfully monitored in many parts of the world using mussels or related bivalve molluscs.[1] These filter-feeding animals can concentrate chemicals in their tissues to several thousand times the levels in the water column.

One of the most successful ways of quantifying the impact of chemicals on bivalve molluscs is to determine their energy balance (termed Scope for Growth; SFG) using whole-organism physiological measurements. In locations where there is a single major source of contamination such as an oil terminal, linking adverse effects on SFG to levels of bioaccumulated contaminants is relatively straightforward.[2] However, when such studies are carried out over a scale such as that of the entire UK North Sea coast[3] the number and diversity of contaminant sources makes cause–effect linkages much more difficult to establish. An essential prerequisite of achieving this linkage is to have precise quantitative information about how the individual accumulated chemicals detected in mussel monitoring programmes influence SFG. Often the most abundant contaminants detected are aliphatic and aromatic hydrocarbons which are released into the environment as a consequence of fossil fuel burning and the use of petroleum products. Their impact on SFG is sufficiently well understood that Quantitative Structure Activity Relationships (QSARs) have been established for the key SFG parameter, feeding rate.[4,5] The objective of the study described in this paper was to determine the

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responsiveness of mussel SFG to body burdens of neurotoxic pesticides representing the major classes of these compounds in current use, and to interpret the toxicological significance of the data in the context of the established hydrocarbon non-specific narcotic (or baseline toxicity[4,5]) QSARs. The environmental impact of neurotoxic pesticides has been of great concern for many years because of their potency and inevitable release into the environment, particularly when used in agriculture.[6] Compounds of this type always feature strongly in priority pollutant lists[7,8] and despite restrictions on the use of many of the more stable organochlorine compounds, these pesticides are still detected in the tissues of mussels collected from coastal sites.[1,3,6,9]

1.1 Choice of contaminants

The following compounds were chosen for study to represent the major neurotoxic pesticide classes which are known environmental contaminants at the present time.

1.1.1 Organochlorine compounds

Lindane (gamma-HCH) and endrin were selected, the former because it is still in agricultural use in the UK[10–12] and the latter because it is a representative of the commonly occurring cyclodiene group, and comparative physiological data for its effects on fish are available.[13] These compounds are thought to be toxic primarily because they inhibit the binding of the neurotransmitter gamma-aminobutyric acid (GABA) to its receptor sites in the central nervous system.[14]

1.1.2 Organophosphate and carbamate pesticides

These extensively used[6,10,11,15] chemicals inhibit the enzyme acetylcholinesterase (AChE), the correct functioning of which is essential for the transmission of nerve signals, particularly at neuromuscular junctions.[16] Many organophosphate pesticides require oxidative metabolic activation in the target organism to achieve full potency.[16] Since the capacity of mussels to oxygenate foreign compounds is limited,[17] we chose to study dichlorvos, a direct-acting (already oxygenated) compound of considerable interest in the marine environment since it is used in salmon farming to remove lice from the fish.[18]

Carbaryl was selected to represent carbamates since it is applied extensively in the UK and elsewhere,[6,10,15] has been proposed as an alternative to dichlorvos in fish farming,[19] and because of its hydrophobic nature (log octanol/water partition coefficient (log K_{ow}) 2·36), significant bioaccumulation by mussels is predicted.[5,20]

The inhibition of AChE activity in organisms exposed to these compounds can be readily measured.[18,21,22] We have therefore assayed the activity of this enzyme in the gills of mussels exposed to dichlorvos and carbaryl in an attempt to establish a mechanistic link between physiological response and enzyme activity.

1.1.3 Pyrethroids

These compounds are thought to block sodium channels which are essential for transmission of signals along nerve cells.[14,23] Two classes are recognised, based on symptoms of poisoning in mammals; they are termed Type I and Type II. There is evidence that the Type II compounds also inhibit the normal functioning of GABA receptors.[14] Quantitatively the most important pyrethroid used in UK agriculture is cypermethrin,[11] a Type II compound. The solubility of this compound in fresh water is 5–10 μg litre^{-1} [24] and seawater solubility under the conditions of our experiments would be approximately half this value. This causes practical difficulties in our solution-based exposure system (see Section 2) so flucythrinate (aqueous solubility 49 μg litre^{-1}), a close structural analogue and Type II pyrethroid was selected for study. Though not used in the UK, this compound is applied extensively in the USA and elsewhere. A single experiment was also carried out with permethrin, a Type I pyrethroid used in the UK for mothproofing.[25]

Previous research has demonstrated that the most contaminant-sensitive parameters in the SFG determination are feeding rate and respiration rate.[3] However, for the neurotoxins tested, only the feeding rate changed significantly at low toxin concentrations. At high concentrations of some of the test compounds, the ventilation rate of the mantle cavity became so low, with the valves (shells) almost closed, that respiration declined.[26] Since this was clearly an indirect effect of the toxicant, the respiration data will not be discussed in this paper.

2 EXPERIMENTAL METHODS

2.1 Chemicals

Solvents were obtained from Fisons Scientific Equipment (now Fisher Scientific UK) and were HPLC or Distol grades as appropriate. The pesticides were obtained from the following suppliers (purity is in parenthesis). Flucythrinate (99·5%) and permethrin (97%) from Promochem; lindane (99·7%) from the Laboratory of the (UK) Government Chemist; alpha-hexachlorocyclohexane (99%) from Aldrich; carbaryl (98%) and dichlorvos (99%) from Alltech. Physicochemical data were obtained from the literature.[27–29]

2.2 Collection and maintenance of mussels (*Mytilus edulis* L.)

Mussels with a shell length of between 35 and 40 mm were collected from Bull Hill at the mouth of the river

Exe in Devon, UK. Barnacles and other epibionts were removed from the shells by scraping, and the clean mussels held in recirculating seawater at 15°C and 33 ppt salinity with an artificially produced tidal regime which aerially exposed the mussels for two periods of 2·5 h per day.[4] They were fed *via* a peristaltic pump with a culture of the alga *Isochrysis galbana* (Parke) at a rate equivalent to 8 mg dry algal mass per animal per day. The mussels were allowed to acclimate to laboratory conditions for seven days before use in experiments. The seawater used in the holding aquarium and during pesticide exposure experiments was collected from between five and 10 miles offshore to the south of Plymouth.

2.3 Exposure of mussels to toxicant

The experimental procedures used were similar to those described in previous publications.[4,5] Either 16 or 26 (AChE inhibitor experiments) mussels were held at 15°C in a 20-litre round-bottomed reaction vessel containing 18 litres of a solution of the test compound in filtered offshore seawater (salinity 33–35 ppt). The animals were fed continuously with a culture of the unicellular alga *I. galbana* at the rate of approximately 0·1 to 0·15 mg dry weight of algae per animal per hour. For the majority of the pesticides studied, test solutions were prepared by adding pesticide dissolved in acetone (1·1 ml) to 22 litres of filtered seawater held in a glass aspirator.[5] The solutions in the exposure vessels were replaced every 24 h. For carbaryl the solvent used was ethanol (acetone interfered with the analysis of carbaryl in seawater), and at the highest concentration tested the volume of solvent used had to be increased five-fold to achieve a true solution in the ethanol. Each set of feeding rate determinations included an appropriate solvent control group of mussels. The concentration of solvent used had no detectable effect on the feeding rate of mussels when compared to mussels exposed to seawater without solvent addition.

Pyrethroids are extremely hydrophobic and readily lost from aqueous solution by absorption onto glass surfaces. For this reason acetone solutions were added directly to the water surrounding the mussels in the round-bottomed reaction vessels. Prior to addition of the acetone solution, the water in the vessels was stirred rapidly to create a vortex which efficiently dispersed the chemical. This was particularly important in experiments carried out with pyrethroids at above their aqueous solubilities, since emulsions were produced. The pyrethroids (dissolved in 500 μl of acetone) were added daily to each vessel after the water was changed.

In order to enable a steady state to be approached between aqueous and mussel tissue concentrations, exposure experiments with the very hydrophobic compounds lindane, endrin, flucythrinate and permethrin were continued for seven days (168 h) and for the more hydrophilic dichlorvos and carbaryl, for three days (72 h; see Ref. 30). Concentration–response curves for each pesticide were generally obtained using mussels from a single field collection. However, the carbaryl curve includes data from experiments conducted two years apart (see Section 3). When the data are expressed as percentage of control values, their is no evidence from this experiment of significant batch-to-batch variability in the response of Exe mussels to aqueous solutions of carbaryl. This is consistent with observations made with hydrocarbons using mussels taken from a single site over many years.[4,5]

2.4 Measurement of feeding rate

The feeding rate of the control or pesticide-exposed mussels was determined by measuring the rate at which individual mussels cleared a suspension of the unicellular alga *I. galbana* from seawater (or pesticide solution in seawater). This measurement is therefore often described as the clearance rate (CR). It was determined on 16 individual mussels from each pesticide or control condition using methods described in detail in our earlier publications.[4,5,31] In summary, each mussel was held in a 2-litre volume of filtered seawater (or pesticide solution in filtered seawater) contained in a 2-litre tall-form beaker. The water was stirred by a magnetically driven Teflon-coated stirrer bar. The mussels were allowed 30 min to settle in the beakers and a standard aliquot of algae added. The concentration of algae in each beaker was determined by means of a Coulter particle counter at 20-min intervals to a total time period of 80 min. These counts were used to calculate the clearance rate. Values obtained from toxicant-exposed mussels were expressed as a percentage of the mean control value and concentration–response curves created. The clearance rate EC_{50} values were derived from these curves after fitting either a linear or logarithmic model to the data as appropriate.[4] A minimum of four exposure concentrations plus a control feeding rate determination was used to establish each EC_{50} value. The precision typically obtained with the EC_{50} determinations is indicated in our previous publication.[4]

2.5 Measurement of gill acetylcholinesterase activity

The AChE activity was measured on the 10 additional animals exposed to dichlorvos and carbaryl. The enzyme activity was measured in phosphate buffer (pH 8·0) using a microplate reader as described in the literature.[18,21,22] This method uses acetylthiocholine iodide as substrate and dithiobisnitrobenzoate to develop the colour assayed. Gills were homogenised immediately following dissection using a glass hand-operated tissue

homogeniser and the homogenate then centrifuged at 13 000*g* for 10 min. Assay reagent volumes and concentrations were optimised for maximum sensitivity.

2.6 Chemical analysis

The concentration of the pesticides in the water to which the mussels were exposed was measured to establish the extent to which the desired concentrations had been achieved and maintained. Mussel tissues were analysed at the end of the experiments to determine the degree of bioaccumulation of the pesticides and in order to make quantitative body-burden/effects links.

2.6.1 Analysis of water

Carbaryl was analysed by direct UV spectrophotometry of the seawater solutions using a wavelength of 280 nm. Quantification was achieved by comparison with standard solutions prepared in seawater. At all concentrations throughout the range tested, levels in reaction vessels and beakers measured following exposure of the mussels were >75% of the nominal concentrations. Nominal concentrations have therefore been used in all presentation and analysis of the carbaryl data.

Lindane and endrin were extracted from 1-litre water samples into 4 ml of 2,2,4-trimethylpentane or iso-hexane by steam distillation for 2 h[32] (see also Refs 33, 34). The extracts were dried by freezing out the water at −17°C and analysed by gas chromatography (GC) using a 15 m × 0·32 mm ID SE-52 quartz capillary column fitted with a 2 m × 0·53 mm ID quartz retention gap, helium carrier gas and a flame-ionisation detection system. The GC oven was programmed for lindane analysis from 90°C to 244°C at 11°C min^{-1} and for endrin 70°C (held 1 min) to 260°C (held 5 min) at 20°C min^{-1}. The concentrations of lindane and endrin in the reaction vessels and beakers at the end of the exposure periods were typically >65% of the nominal concentrations except at the highest concentrations used (which were close to the solubility limit in seawater[27]), where measured levels dropped to 50% of nominal. Consequently the toxicological data for these compounds has been calculated using measured concentrations.

The pyrethroids were extracted from 1-litre water samples held in 1-litre volumetric flasks by shaking with three successive 3-ml aliquots of 2,2,4-trimethylpentane. The combined extracts were dried by freezing out the water and analysed by GC on a 5 m × 2 mm ID glass column packed with 3% SE-30 on Chromosorb (100–120 mesh) and run isothermally at 210°C with a nitrogen gas flow rate of 40 ml min^{-1}. Detection was by electron capture. Measured concentrations in water in the reaction vessels and beakers at the end of the exposure period were less than 50% of the nominal values. The measured values were therefore used for data analysis.

Dichlorvos was extracted from 1-litre water samples by shaking with three successive aliquots of dichloromethane (25, 5 and 5 ml, cf. Ref. 35). The combined extracts were dried over anhydrous sodium sulfate (activated at 500°C), concentrated by evaporation under a stream of nitrogen, then analysed by GC on the same column as used for lindane and endrin linked to a flame photometric detector set to selectively measure phosphorus. The GC oven was programmed from 100 to 220°C at a rate of 10°C min^{-1}. Although dichlorvos was detected in the exposure water using this procedure, the method was not sufficiently sensitive to measure the concentrations of this compound reliably at the two lowest exposure levels used. Nominal concentrations were therefore used for all data analysis. Dichlorvos is stable in seawater over a 24-h period.[18]

2.6.2 Analysis of mussel tissues

After completing the physiological measurements, the mussels were divided into two groups of eight, the tissues dissected out and each group of eight stored at −17°C in a solvent-cleaned glass jar. Before analysis each group was homogenised at 0°C.

Lindane and endrin were extracted from the homogenised tissue samples (6 g wet weight) by steam distillation into 4 ml of 2,2,4-trimethylpentane or iso-hexane. The endrin-containing samples were saponified for 2 h before steam distillation[32] (see also Ref. 36). Lindane is unstable in strong alkali, so the samples containing this compound were steam distilled without the addition of acid or alkali. The extracts were dried and analysed by GC as described for water analysis.

Dichlorvos and carbaryl were extracted using a modified version of the multi-residue procedure developed by Luke *et al.*[37,38] (see also Ref. 39). The tissues (5 g) were homogenised in acetone + acetic acid (99 + 1 by volume; 10 ml) at 0°C, then the solvent filtered off through a sintered glass filter. The tissue was re-extracted with dichloromethane (2 × 10-ml aliquots) and the combined acetone/dichloromethane/acetic acid extracts partitioned against water (5 ml) containing 0·7 g of sodium chloride. The dichloromethane (i.e. lower) layer was removed and the aqueous layer re-extracted with a further 10 ml of dichloromethane. The combined dichloromethane layers were dried over anhydrous sodium sulfate, then concentrated under a stream of nitrogen to 2 ml. Interfering materials were removed by chromatography on an activated (at 170°C) Florisil (3·5 g) column topped with 2 g of anhydrous sodium sulfate. The column was rinsed with iso-hexane + acetic acid (99 + 1 by volume; 20 ml) then the sample mixed with 20 ml of iso-hexane + dichloromethane + acetic acid (89 + 10 + 1 by volume) and loaded onto the column. A first fraction (a) of 25 ml of solvent was collected then further fractions as follows: (b) iso-hexane + dichloromethane + acetic acid (84 + 15 + 1 by volume; 20 ml) and (c) ethyl acetate

(10 ml). The fractions were reduced to 10 ml under a stream of nitrogen and analysed on the same GC system as used for dichlorvos extracted from water. Most of the dichlorvos was eluted in fractions (a) and (b). This method was not sensitive enough to detect dichlorvos in tissues at the lowest two exposure levels tested. It was therefore applied only to tissue analysis of mussels exposed to dichlorvos at 0·3 mg litre^{-1}. This exposure concentration just exceeded the WEC_{50} (water concentration-based EC_{50}) for feeding-rate inhibition by dichlorvos, so could be used to estimate the TEC_{50} (tissue concentration-based EC_{50}).

The dried carbaryl extract was cleaned up on a Florisil column in a similar way to dichlorvos, though the eluting solvents for the later fractions were modified. Fraction (b) was eluted with dichloromethane + ethyl acetate + acetic acid (98 + 1 + 1 by volume, 26 ml); fraction (c) with dichloromethane + ethyl acetate + acetic acid (89 + 10 + 1, by volume, 10 ml) and fraction (d) with 10 ml more of the same solvent. Carbaryl was largely eluted (>90%) in fraction (b). The fractions were reduced in volume under a stream of nitrogen, transferred to acetonitrile, then analysed by high performance liquid chromatography on a Hypersil ODS column (250 × 4·5 mm) eluted with a solvent gradient starting at acetonitrile + water + acetic acid (50 + 50 + 1 by volume[40]) and increasing to 100% acetonitrile. Detection was by fluorescence (excitation wavelength 260 nm, emission wavelength 340 nm).

Flucythrinate was analysed by a modification of the method of Schimmel *et al.*[41] Tissues (5 g) were homogenised at 0°C with acetonitrile (4 × 5 ml aliquots), the extracts filtered off, bulked, partitioned between aqueous sodium sulfate solution (2 g litre^{-1}; 75 ml) and methyl *tert*-butyl ether + iso-hexane (1 + 1 by volume; 3 × 10 ml aliquots), dried at −17°C, then cleaned up by passage through an activated (3 g) Florisil column topped with anhydrous sodium sulfate (2 g). The final eluting solvent was iso-octane + 2-propanol (9 + 1 by volume; 5 ml). The cleaned-up extract was reduced in volume under a stream of nitrogen and the flucythrinate concentration determined by GC as described for water analysis.

The analytical procedures for water and tissues were tested before use by spiking experiments. Recoveries for the different procedures ranged from 80 to 95%. All analyses of samples from experiments were carried out at least in duplicate and repeated if duplicate values fell outside the range of ±10% from the mean. Quantification was by external standard calibration except for lindane analysis for which alpha-hexachlorocyclohexane was used as an internal standard. External standard solutions encompassing the concentration range of the analyte present in the samples being analysed were injected at regular intervals throughout the working day. Response factors were calculated and any deviations of more than 5% from previous results were checked by repeat injections. Analyte concentrations in the samples were calculated from an average response factor derived from three or more standard injections. No correction was made for incomplete recovery of compounds quantified by external standard calibration.

3 RESULTS AND DISCUSSION

3.1 Organochlorine compounds

Lindane was toxic to mussels, reducing the feeding rate to zero at a measured aqueous concentration of 1·5 mg litre^{-1} (3 mg litre^{-1} nominal, Fig. 1(a)). This concentration is probably close to the solubility limit of lindane in seawater at 15°C, since values ranging from 2 to 7·3 mg litre^{-1} have been reported for its solubility in pure water at 20 to 25°C.[27] A similar concentration–response relationship is obtained when the data are presented on a body burden basis (Fig. 1(b)).

Though lindane can clearly have a severe adverse effect on the feeding rate of mussels, these data alone cannot provide evidence that it is functioning as a neurotoxin. Research carried out on numerous different types of organism during the last century has demonstrated the existence of a mode of toxic action common

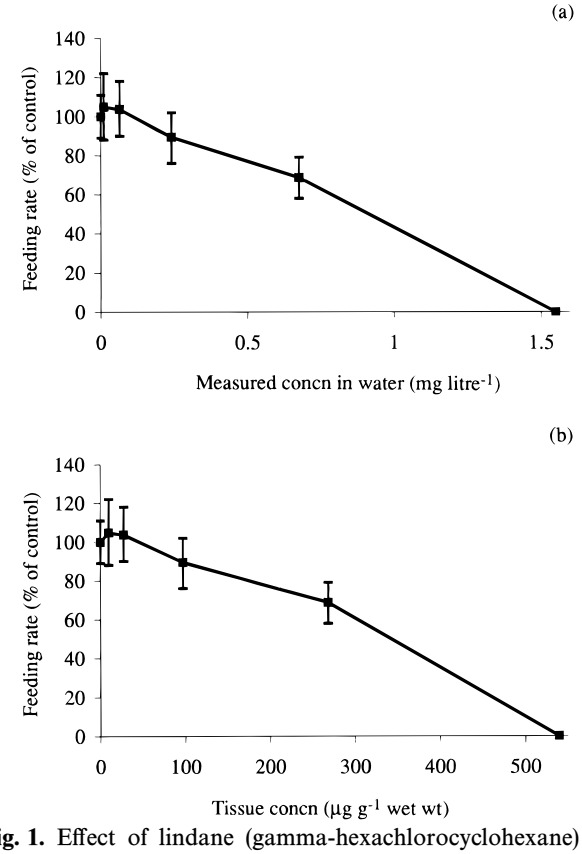

Fig. 1. Effect of lindane (gamma-hexachlorocyclohexane) on the feeding rate of *Mytilus edulis* (seven-day exposure). Error bars in this and subsequent figures represent 95% confidence intervals.

to many organic compounds.[42] This has been described as non-specific narcosis, and represents a baseline or minimum toxicity which can be expected to be possessed by compounds having properties defined by a certain physicochemical 'window'. The aquatic toxicity data for non-specific narcotics can be represented by QSARs having only one physicochemical variable, frequently K_{ow}. Using such QSARs, the narcotic toxicity of a compound of known K_{ow} can be predicted and compared with actual experimental data. A convenient way of expressing this relationship is to calculate a ratio between the predicted and observed values.[43] Toxicity greater than the narcotic prediction is taken to indicate that the compound of interest has an *additional*, more specific, mode of action. This method of data evaluation has been applied to our results, using our previously established non-specific narcotic mussel feeding rate QSAR[4,5] as the comparative baseline. This QSAR was derived from data obtained with 21 hydrocarbon and substituted hydrocarbon compounds. Since these data were collected over several years and at different times of the year, any variability due to seasonal factors will be included in the overall variability of the QSAR. In our published hydrocarbon studies and the studies on pesticides reported in this paper, the concentration–response plots for each compound were used to determine the concentration required to reduce feeding rate to 50% of the control value. These feeding rate EC_{50} values are the basis for the toxicological comparisons made.

The data are summarised in Tables 1 and 2 which also include figures derived from the work of Widdows and Page[44] on another biocide of concern in the marine environment, the antifouling compound tributyltin (TBT) and its degradation product dibutyltin (DBT). When toxicity is expressed on the basis of aqueous exposure concentrations (WEC_{50}; Table 1), lindane appears to be no more toxic than a non-specific narcotic of comparable hydrophobicity. Based on our previous research,[5] the body-burden of a narcotic compound required to reduce feeding rate to 50% of the control value was 224($\pm$ 89) (SE, $n = 14$) μmol kg^{-1}.

TABLE 1
Effect of Pesticides on Feeding Rate of Mussels: Toxicity Expressed as Concentration in Water

Compound	*Log* K_{ow}	EC_{50} *Narc.*	(μ*mol litre*$^{-1}$) *Obs.*	EC_{50} *Narc./* EC_{50} *Obs.*
Lindane	3·61	4·41	3·58	0·81
Endrin	4·56	0·79	0·12	6·68
Carbaryl	2·36	41·6	33·9	0·81
Dichlorvos	1·16	359·1	0·45	798·0
Tributyltin[a]	3·80	3·13	0·0006	5690·9
Dibutyltin[a]	1·49	198·5	0·33	603·4

[a] Data from Ref. 44.

TABLE 2
Effect of Pesticides on Feeding Rate of Mussels: Toxicity Expressed as Concentration in Mussel Tissue

Compound	TEC_{50} *Obs.* (μ*mol* kg^{-1} *wet wt*)	EC_{50} *Narc.*[a]/ EC_{50} *Obs.*
Lindane	1030	0·2
Endrin	260	0·9
Carbaryl	50	4·5
Dichlorvos	2	112·0
Tributyltin	5	46·7
Dibutyltin	39	5·8

[a] TEC50 narcosis = 224($\pm$ 89)(SD, $n = 14$) μmol kg^{-1} wet wt (from Ref. 5).

Our measured tissue concentration EC_{50} (TEC_{50}) for lindane was greater than this (Table 2), indicating toxicity *less* than the narcotic baseline. The concept that narcosis occurs when a constant body-burden of chemical is reached has been established for many years and is supported by experimental data.[42,45,46] Although exceptions to this general rule do occur, the literature provides few clues as to why the sublethal toxicity of lindane towards mussels should be atypical.

One possible explanation for such a result is that the lindane was compartmentalised in some toxicologically unavailable form within the organism. An indication that this could have occurred is given by the data presented in Table 3, which show the predicted and observed bioconcentration factors (BCF) for the compounds studied. The BCF is the ratio of the concentration of compound in mussel tissues to concentration measured in the exposure water. Two predicted values are given for lindane, the first derived from our published QSAR,[5] the second from a QSAR published by Geyer *et al.*[20] The latter QSAR gave a much closer fit to the observed data, probably because it was derived using a structurally diverse group of compounds which were predominantly organochlorines. Our QSAR was

TABLE 3
Bioconcentration of Pesticides into Mussels: Comparison of Observed and Predicted Bioconcentration Factors

Compound	*BCF*[a] *Predicted*	*Observed*	*Predicted/ Observed*[a]
Lindane	40 [195]	343	0·1 [0·6]
Endrin	386 [1500]	2274	0·2 [0·7]
Carbaryl	2·1 [16]	2·4	0·9 [6·7]
Dichlorvos	0·1 [1·5]	1·1	0·1 [1·4]
Tributyltin	64 [283]	10 000	0·006 [0·028]
Dibutyltin	0·3 [3·0]	110	0·003 [0·027]

[a] Figures given in parentheses are based on Geyer *et al.*[20]

derived largely with hydrocarbons; Pruell *et al.*[47] showed that under identical exposure conditions, the BCF of polychlorinated biphenyls (PCBs), which like lindane are chlorinated hydrocarbons, was greater than that of unsubstituted hydrocarbons. Furthermore, the slope and intercept of their hydrocarbon-based QSAR was, like ours, greater than that of Geyer's[20] equation. Hawker and Connell[30] have also published an organochlorine-pesticide-dominated BCF QSAR using data obtained with several filter-feeding bivalve mollusc species. Though the slope closely resembles that of Geyer's equation,[20] the predicted BCF of lindane (based on a log K_{ow} of 3·61) was only 64. Despite this difference, both equations predict lower bioaccumulation than we observed, though the field-derived BCF for lindane of 350 cited by Geyer[20] very closely resembles the value obtained in our experiments. This may reflect the fact that mussels in our experiments were fed digestible algal food which may have enhanced uptake and sequestration of lindane through the increased filtering activity produced in response to food and through the uptake of lindane absorbed on the algae. Recent research on *Daphnia magna* Straus[48] has shown that the presence of algae during toxicity tests with the moderately hydrophobic compound 3,4-dichloroaniline (log K_{ow} 2·69) can reduce the dissolved concentration of this compound by between 5 and 15%. The concentrations of algae used in the *Daphnia* experiments were much greater than during the exposure phase of our experiments with mussels. Therefore, uptake by partitioning from the aqueous phase was likely to be the dominant mechanism in our experiments. This conclusion is of some importance in the interpretation of results, since uptake experiments where particulate-absorbed material may have a role are actually measuring bioaccumulation factors rather than bioconcentration factors.

The higher-than-expected bioaccumulation of lindane in our experiments, combined with unexpectedly low toxicity does indicate that some of the pesticide may be sequestered in a toxicologically unavailable form. A likely location is lipid reserves associated with the seasonal gametogenic cycle. The data presented in this paper were obtained at different times of the year using mussels in various stages of the gametogenic cycle. Seasonal changes in lipid levels are known to influence the bioaccumulation of organochlorine compounds by mussels,[49,50] and in fish[51] and in terrestrial animals,[52] differences in lipid levels have been shown to influence the response of the organisms to toxicants.

Despite these sources of variability, the data obtained in our experiments indicate that lindane is no more toxic than a narcotic compound of comparable hydrophobicity, so does not act as a neurotoxin towards the ciliary feeding process of mussels. This suggests that GABA receptors are not involved in the control of ciliary feeding. Lindane and other organochlorine pesticides are known to induce lipid peroxidation in mammals, potentially enhancing their toxicity.[53,54] Clearly this biochemically mediated mode of toxic action does no occur in mussels or did not cause sufficient damage during the timecourse of our experiments to affect feeding rate adversely.

The data from the experiments with endrin, when plotted, produced concentration–response curves of similar form to those shown in Fig. 1 for lindane. The WEC_{50} determined from these plots indicated greater toxicity than would be predicted for a narcotic of the same K_{ow} (Table 1). However, by expressing the data on a tissue residue basis (TEC_{50} ; Table 2) endrin can be shown to be behaving like a narcotic towards mussel ciliary feeding activity, so, like lindane, there is no evidence for neurotoxicity. The apparent enhanced toxicity of aqueous solutions was due to the poor predictive capability of our hydrocarbon-based QSAR for bioconcentration of organochlorine pesticides (Table 3). The BCF value for endrin presented in parenthesis in Table 3 was calculated using the QSAR published by Geyer *et al.*[20] The actual BCF value (1920) used by Geyer in the construction of his QSAR was reported originally by Ernst,[55] and is 84% of the value we obtained, well within the difference which could be attributed to experimental factors.

In fish, endrin has been shown to induce a physiological response syndrome which includes an elevated respiration rate.[13] There was no evidence for such a response in our experiments on mussels.[26] Our data for lindane and endrin therefore clearly indicate that these compounds are no more toxic to mussels than narcotic chemicals of comparable physicochemical properties. This conclusion is supported by literature reports of long-term exposure experiments which show that bivalve molluscs are rather insensitive to organochlorine pesticides.[56,57]

3.2 Organophosphate and carbamate pesticides

The organophosphate compound dichlorvos reduced the feeding rate of mussels to approximately 40% of the control value at a nominal aqueous concentration of 300 μg litre^{-1}, but a further increase in concentration to 1000 μg litre^{-1} did not cause any additional depression of feeding rate (Fig. 2). This somewhat unusual concentration–response curve gave rise to a WEC_{50} value (Table 1) indicating that dichlorvos is considerably more toxic than a narcotic of similar physicochemical properties. Because of the low level of bioconcentration of this rather hydrophilic compound and the poor sensitivity of the analytical procedure used, a complete body-burden–response curve was not obtained. The TEC_{50} given in Table 2 was obtained by extrapolation from body-burden measurements made

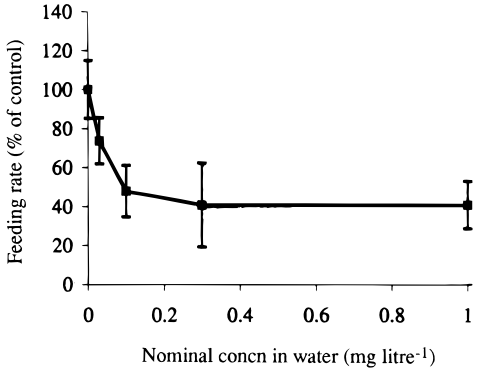

Fig. 2. Effect of dichlorvos on the feeding rate of *Mytilus edulis* (three-day exposure).

on mussels exposed in an additional experiment to 300 μg litre^{-1} of dichlorvos. This experiment produced a feeding rate reduction to 65% of the control value. The additional feeding-rate data and the body-burden data confirmed the elevated toxicity of dichlorvos. The measured BCF (Table 3) was consistent with the QSAR prediction made from the Geyer equation.[20] Although dichlorvos inhibits feeding rate, mussels contaminated with this compound *appeared* to be very active, as the mantle edges were fully extended. However, the tone of the adductor (shell closing) muscles seemed poor, giving the impression of a relaxed state. These observations are in marked contrast to the response of mussels to narcotic chemicals such as hydrocarbons, which cause the animals to retract their mantle edges and restrict the opening of the siphons and valves in a concentration-dependent manner. These behavioural responses suggest that the dichlorvos has a neurotoxic effect on the mussels. The work of several other research groups strongly supports this conclusion.

McHenery *et al.*[58,59] investigated the impact of applying dichlorvos to eliminate ectoparasitic copepods from salmon farmed in Scottish sea lochs. In laboratory experiments they were unable to detect any effect of dichlorvos on the efficiency with which mussels filtered bacteria from the water column, but bacteria are poorly retained by the gills, whereas there is 100% retention of algae.[60] However, McHenery *et al.*[58] did obtain a clear concentration–response relationship between time taken for mussels to close and dichlorvos exposure. The 24-h WEC_{50} for closure impairment was 1690 μg litre^{-1}. Thain *et al.*[61] reported a 72-h WEC_{50} value for foot relaxation in limpets (*Patella vulgata* L.) of only 12·8 μg litre^{-1}, and growth was inhibited in juvenile oysters (*Crassostrea gigas* Meusch.), clams (*Venerupis decussata*) and mussels (*M. edulis*) exposed for >40 days to dichlorvos concentrations similar to those producing effects in our experiments. Dichlorvos and another AChE-inhibiting organophosphate pesticide, malathion, have been shown to alter the periodic activity of freshwater mussels (*Anodonta* species[62]), and field mortality of the freshwater mussel (*Elliptio steinstansana*) in the USA has been attributed to AChE-inhibiting pesticides.[63]

Figure 3 clearly demonstrates that AChE activity in the gills of mussels exposed to dichlorvos in our experiments is reduced in a concentration-dependent manner down to zero at a nominal aqueous concentration of 100 μg litre^{-1}. The WEC_{50} for this response is 3 μg litre^{-1}; McHenery *et al.*[58] reported a 24-h WEC_{50} for AChE inhibition of 3·6 μg litre^{-1}. The form of the AChE inhibition curve is not the same as that for feeding rate (Fig. 2), so AChE does not appear to control feeding activity directly in a linear fashion. The precise role of acetylcholine in the control of gill activity is uncertain at the present time,[64] but acetylcholine and AChE inhibitors are known to influence the activity of molluscan mussels,[65,66] so the effect on feeding rate may be caused by changes in the tone of muscles along the gill filaments. Widdows and Ward (unpublished) observed changes in the shape of mussel gill filaments in response to acetylcholine; these changes were thought to be the result of contraction of the gill musculature.

Carbaryl (Fig. 4) reduces feeding rate in a simple concentration-related manner. The data shown in Fig. 4(a) were derived from two separate experiments carried out two years apart; this indicates how reproducible the feeding rate measurements can be. The shape and slope of the concentration–response curve (similar to that for lindane in Fig. 1) and the WEC_{50} (Table 1) suggest that carbaryl is acting simply as a narcotic, though when toxicity is expressed in terms of tissue concentration (Table 2) there is evidence of a small enhancement over narcotic behaviour. The uncharacteristically poor predictive capability of the Geyer *et al.*[20] equation for the BCF of carbaryl (Table 3), may indicate that this compound is unstable in mussels. Figure 5 shows that, within the range of concentrations shown to have an adverse effect on feeding rate, the activity of AChE is severely reduced. However, as in the case of dichlorvos, the form of the AChE-concentration–response curve is different from that of feeding rate, suggesting that the link between the two parameters is not simple. It is

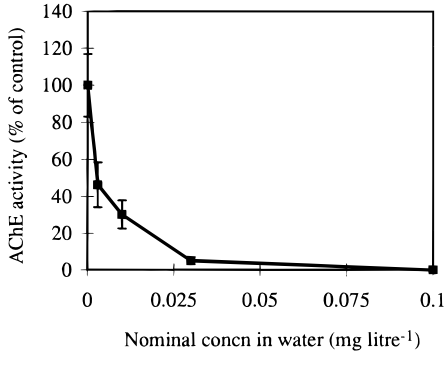

Fig. 3. Effect of dichlorvos on acetylcholinesterase activity in the gill of *Mytilus edulis* (three-day exposure). Activity in gills of control (unexposed) mussels was 12 000(±1570) (±95% c.i.) units mg^{-1} protein.

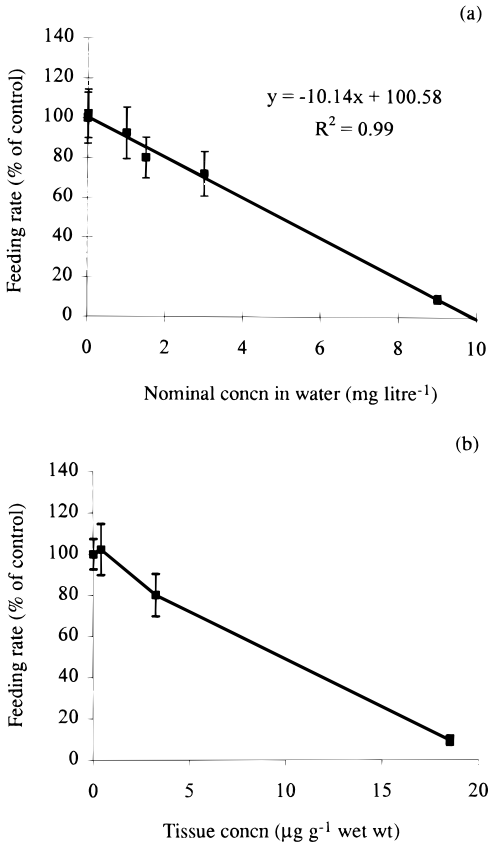

Fig. 4. Effect of carbaryl on the feeding rate of *Mytilus edulis* (three-day exposure).

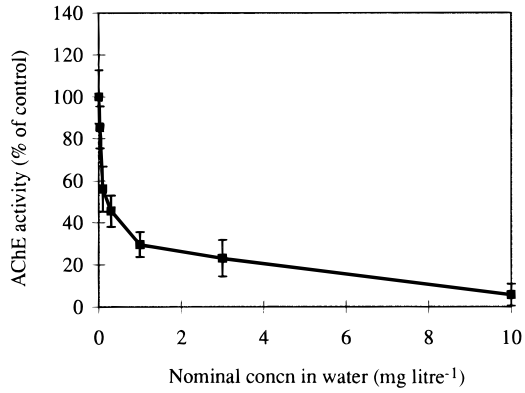

Fig. 5. Effect of carbaryl on acetylcholinesterase activity in the gill of *Mytilus edulis* (three-day exposure). Activity in gills of control (unexposed) mussels was 10 700(±1350) (±95% c.i.) units mg^{-1} protein.

instructive to note that the WEC_{50} for AChE inhibition by dichlorvos (3 μg $litre^{-1}$; 0·014 μmol $litre^{-1}$) is much lower than for carbaryl (200 μg $litre^{-1}$; 0·99 μmol $litre^{-1}$) despite the higher level of bioaccumulation of carbaryl. Even animals such as fish and crustaceans, which are highly sensitive to organophosphate AChE inhibitors, are much less sensitive to carbamates.[41,67,68] It seems likely therefore, that the toxicity of carbaryl to mussel-feeding activity is dominated by its direct narcotic effect, with only a small neurotoxic contribution due to its relatively weak AChE-inhibiting activity. This view is supported by studies which showed that exposure of mussels for four to seven days to carbaryl at 10 mg $litre^{-1}$ killed only 10–30% of the animals.[69]

3.4 Pyrethroids

Mussels were first exposed for seven days to flucythrinate at a nominal concentration of 40 μg $litre^{-1}$ (0·09 μmol $litre^{-1}$), which is close to the solubility limit in seawater.[24,41] The mean measured concentration at the end of the exposure period was 14(±6) μg $litre^{-1}$ (mean = 0·03 μmol $litre^{-1}$). This concentration had no effect on feeding rate (Table 4). The body-burden of flucythrinate achieved under these conditions was 12·1 μg g^{-1} wet weight (27 μmol kg^{-1}) which would only produce a small reduction in feeding rate if the compound was narcotic.[5] In an attempt to produce a response, the exposure concentration was increased 10-fold to 400 μg $litre^{-1}$ (0·9 μmol $litre^{-1}$), a concentration which was clearly above the solubility limit, since an emulsion was formed. The measured concentration at the end of exposure was highly variable, perhaps due to instability of the emulsion, averaging 55(±41) μg

TABLE 4
Effect of Pyrethroids on Feeding Rate of Mussels

Pyrethroid (*Log* K_{ow})	*Nominal concentration in water* (*µg litre*$^{-1}$ (*µmol litre*$^{-1}$))	*Feeding rate* (*% of control*) (± *95% c.i.*)	*Body-burden* (*µg g*$^{-1}$ *wet wt*) (± *range*)
Flucythrinate			
(6·2)	40 (0·09)	99·8 (±6·3)	12·1 (±0·2)
	400 (0·9)	95·1 (±7·8)	203·0 (±10·4)
Permethrin			
(6·5)	400 (1·0)	33·0 (±9·6)	n.m.[a]

[a] n.m. = Not measured.

litre^{-1} (mean = 0·12 μmol litre^{-1}). This procedure led to a bioaccumulation of 203 μg g^{-1} wet weight (450 μmol kg^{-1}). Although some of the flucythrinate measured under these conditions may have been superficially retained emulsion, this tissue concentration is within the range expected to produce detectable narcotic effects on ciliary feeding activity;[5] no such effects were observed. Calculated BCF values based on the aqueous concentrations of flucythrinate measured at the end of the exposure period were 863 and 3691 for the lower and higher exposure levels respectively. QSAR-based BCF estimates of 18 880 and 32 479 were obtained from the equations in Donkin *et al.*[5] and Geyer *et al.*[20] The large disparity between the observed and predicted values indicates that a steady state between flucythrinate in mussel tissue and exposure water had not been reached during the experiment. Longer exposure may therefore have increased bioaccumulation (assuming flucythrinate is not metabolically degraded by the mussels), with the possibility of effects on feeding rate occurring at the higher tissue concentrations theoretically achievable. However, Schimmel *et al.*[41] reported that the steady-state BCF of flucythrinate in oysters (*Crassostrea virginica* Gmel.) was only 2300, and attained in less than 10 days. This suggests that the BCF QSARs in the literature may be inadequate predictors of the bioaccumulation of pyrethroids by bivalve molluscs.

In order to establish whether the absence of toxicity towards mussel feeding efficiency is a general property of pyrethroids, a single seven-day, high exposure concentration (400 μg litre^{-1}; 1·0 μmol litre^{-1}) experiment was carried out with permethrin. This concentration is above the seawater solubility of permethrin (50 μg litre^{-1})[24] so the compound was present as an emulsion. No body-burden measurements were made, but, since the log K_{ow} of this compound is similar to that of flucythrinate (6·5 for permethrin and 6·2 for flucythrinate),[24] a comparable degree of bioaccumulation can be predicted. Exposure to this concentration of permethrin reduced the feeding rate to 33% of the control value (Table 4), which would be equivalent to a WEC_{50} of approximately 300 μg litre^{-1} (0·77 μmol litre^{-1}) if a linear concentration–response curve, typical of the effects of many non-polar narcotics on mussel feeding rate, is assumed. If permethrin (log K_{ow} 6·5) behaved like a narcotic hydrocarbon which fitted the established mussel feeding rate QSAR line,[5] a WEC_{50} of 6·4 μg litre^{-1} (0·016 μmol litre^{-1}) would be predicted. Taking the published seawater solubility (50 μg litre^{-1})[41] as the maximum concentration achievable gives a predicted/observed toxicity ratio of 0·13. If the nominal (emulsion) concentration WEC_{50} of 300 μg litre^{-1} is used for this calculation, the ratio becomes 0·021, indicative of even lower toxicity.

The reason why permethrin is toxic, albeit of low potency and flucythrinate is non-toxic is not clear. Both have aqueous solubilities of 50 μg litre^{-1} (0·13 and 0·11 μmol litre^{-1} for permethrin and flucythrinate respectively), close to the minimum level required to produce narcotic effects on mussel feeding with hydrocarbons,[5] so small differences in the physicochemical properties of the two pyrethroids may be sufficient to alter the balance between toxic and non-toxic behaviour. Permethrin is classified as a Type I pyrethroid and flucythrinate as Type II, but since there is no evidence from our data of specific neurotoxic activity, it seems unlikely that these neurotoxicity-defined classifications would be relevant to the differences in toxicity towards mussel feeding behaviour. Recent research (Wraige, E. J., pers. comm.) has indicated that seasonal factors may influence the response of mussel feeding rate to compounds close to the limits of narcotic toxicity; our experiments with flucythrinate and permethrin were carried out at different times of the year.

Though the data for the two pyrethroids raise interesting mechanistic questions, the key observation is that both compounds are of very low toxicity towards mussel feeding activity. This is consistent with insensitivity demonstrated in growth and mortality studies carried out with both adult and larval stages of other bivalve mollusc species.[24] Varanka[62] has reported that a pesticide formulation containing the pyrethroid deltamethrin alters the periodicity of opening of freshwater bivalves at concentrations far below the LC_{50} for the product. Since the pure pyrethroid was not tested in these experiments and no comparisons were made with responses to non-neurotoxic compounds (e.g. non-specific narcotics) it is difficult to assess the significance of these observations. It is however possible that pyrethroids have some specific effect on the nervous system of bivalves which could be used as an impact monitoring tool.

To date, our experiments on the effects of neurotoxic pesticides on the feeding rate of marine mussels have involved only two compounds to represent each of the major pesticide groups, the organochlorines, the organophosphates and carbamates and the pyrethroids. This data base is too small to establish any precise structure–activity relationships, but some trends are apparent which are consistent with literature reports of the effects of these groups of chemicals on other biological responses of bivalve molluscs.

The organochlorines and the pyrethroids tested were no more toxic to the feeding activity of mussels during seven days' exposure than would be non-specific narcotic compounds of similar physicochemical properties. They had no detectable neurotoxic effects on feeding. Consequently, the concentration of the individual pesticides required to have significant effects on feeding rate far exceeds the levels commonly reported in mussel tissues and water from field sites. Lindane and cyclodiene (usually dieldrin) concentrations in mussel tissues rarely exceed 0·06 and 1·0 μmol kg^{-1} wet weight

respectively[1,3] and levels in heavily polluted UK *rivers* (not seawater-diluted estuaries) are reported up to 0·72 and 0·03 nmol litre^{-1} [70] (cf. Tables 1 and 2). We are not aware of any tissue residue data for pyrethroids in mussels, but concentrations of permethrin and cypermethrin up to 1·0 and 0·4 nmol litre^{-1} respectively have been detected in some UK rivers[70] and permethrin has been detected in sewage sludges (which are often dumped at sea) at concentrations of up to 19 μmol kg^{-1} (dry weight).[71] The environmental concentrations of the organochlorines and pyrethroids are therefore insufficiently high to individually have a significant adverse impact on mussel feeding rate. As nonspecific narcotics, they are, however, likely to have an additive effect in combination with more quantitatively significant anthropogenic contaminants such as hydrocarbons.[42,72]

The low sensitivity of mussel feeding rate (hence Scope for Growth) to these compounds has important implications for the use of mussels (and related bivalve molluscs) to assess their environmental impact. This is perhaps best illustrated by considering the pyrethroids. An Environmental Quality Standard (EQS) of 1 ng litre^{-1} (0·003 nmol litre^{-1}) has been proposed for the protection of marine life from the effects of the pyrethroids cyfluthrin and permethrin which are used in mothproofing.[25] These stringent EQSs are necessary since 96-h LC_{50} values as low as 5 ng litre^{-1} (0·012 nmol litre^{-1}) have been reported for the effect of cypermethrin (a close structural analogue of cyfluthrin) on the shrimp *Mysidopsis bahaia*.[24] Though not as sensitive to pyrethroids as crustaceans, fish would be killed at concentrations which would have no detectable *sublethal* effects on bivalve mollusc feeding rate.[24]

Concentrations of pyrethroids in excess of the EQSs are frequently reported in UK rivers,[70] so may also be present in some estuaries. However, it is firmly established that much of the pyrethroid contamination detected in the environment is absorbed onto sediments and colloids and is not immediately biologically available, so may not be toxic.[24] Therefore, the only satisfactory way to assess the impact of pyrethroids is to employ a pyrethroid-sensitive *in-situ* bioassay. Crustaceans are pyrethroid-sensitive, ecologically and commercially important and practical organisms for the study of sublethal effects. For example Day and Kaushik[73] have shown that the feeding rate of the freshwater crustacean *Daphnia* is very sensitive to pyrethroids and McKenney and Matthews[74] have demonstrated that physiological energetic parameters can be measured in estuarine *Mysids* and are sensitive to a neurotoxic pesticide (fenthion, an organophosphate). These methods are currently being further developed at the Plymouth Marine Laboratory.

Although the tested physiological responses of bivalve molluscs appear to be of little value as indicators of the impact of pyrethroids on species-diverse marine communities, our data and those of other researchers suggest that they have considerable potential for monitoring the impact of potent direct-acting acetylcholinesterase-inhibiting toxicants such as dichlorvos. When expressed on a body-burden basis (Table 2), dichlorvos is more toxic to the feeding activity of mussels than TBT, a compound of particular concern in the marine environment. The high toxicity of TBT expressed on an aqueous concentration basis is due to its enhanced bioaccumulation over that predicted by simple partitioning (Table 3; see also Ref. 75). For a variety of reasons, tissue residue data for organophosphate and carbamate pesticides in mussels are rarely reported. Analysis of this structurally diverse class of chemicals and their (sometimes toxic) degradation products is difficult, a problem accentuated by their physicochemical properties which give rise to low to medium bioconcentration factors. Furthermore, esterase enzymes with the potential to degrade these compounds are widespread and abundant in mussels,[76] probably resulting in a short half-life within the animal.

Despite the instability of organophosphates, inhibition of AChE activity by dichlorvos has been observed in mussels from contaminated Scottish sea lochs.[58,59] Our studies suggest that these reductions in enzyme activity may be linked to moderate reductions in feeding efficiency and scope for growth. The difficulties associated with meaningful chemical analysis of environmental contaminants that are acetylcholinesterase inhibitors increase the importance of the monitoring approach using biological effects. Total organophosphate pesticide levels in the μg litre^{-1} range have been reported for some UK rivers.[70] These total levels frequently exceed the EQS values for individual AChE-inhibiting pesticides which have been established for aquatic environments. Van Zoest and van Eck[77] have applied an *in-vitro* AChE-inhibition assay to estimate total levels of AChE-inhibiting compounds in the Scheldt estuary (Netherlands) and concluded that the levels present in the riverine inputs to the estuary represented a significant 'ecotoxicological risk'. It therefore seems possile that adverse effects due to these compounds may be detectable in some estuarine mussel populations.

4 CONCLUSIONS

Mussels and related bivalve molluscs are probably one of the most successful organisms used in chemical contamination monitoring programmes. They are proving equally successful in biological effects monitoring programmes. However, in both the chemical and biological effects monitoring roles, all organisms have their limitations. The research described in this paper has shown

that mussels are highly sensitive to some neurotoxic pesticides but insensitive to others. This information will enhance our ability to interpret *in-situ* sublethal biological effects observations made using mussels and related bivalve molluscs.

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